CONCEPTION, EMBRYONIC DEVELOPMENT AND CORPUS LUTEUM FUNCTION IN BEEF CATTLE OPEN FOR TWO CONSECUTIVE BREEDING SEASONS

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Received for publication: April 15, 1983
Accepted: October 26, 1983

ABSTRACT

High-fertility (control cows) and low-fertility (cows and heifers not pregnant after two consecutive breeding seasons -- twice-open) cyclic bovine females were treated with a single injection of 1000 IU of human chorionic gonadotropin (HCG) or 100 μg of gonadotropin releasing hormone (GnRH) to enhance and/or hasten corpus luteum formation and progesterone secretion, and improve conception rate in the low-fertility females. Hormone treatments were administered to 38 parous control cows, 34 twice-open parous cows, and 27 twice-open nonparous heifers immediately after natural mating by a fertile bull. Blood samples were collected on Days 3, 6, 9, 12, and 18 after mating for determination of systemic progesterone concentrations. Pregnancy rate at necropsy approximately 33 days after mating (range 31-37) was higher in control cows (73.0%) than in twice-open cows (48.4%; $P<0.05$) or twice-open heifers (34.6%; $P<0.01$). Pregnancy rate was not affected by the HCG or GnRH treatment. The HCG treatment increased plasma progesterone concentrations in twice-open heifers but not in control or twice-open cows. Progesterone was unaffected by the GnRH treatment. Systemic progesterone concentrations were higher in control than in twice-open females but did not differ between pregnant and nonpregnant females of Days 3, 6, 9 and 12 after mating. Enhanced gonadotropin stimulation at estrus by injection of either HCG or GnRH did not increase pregnancy rate or systemic progesterone concentrations (except in HCG-treated twice-open heifers) in low- or high-fertility females. Lower pregnancy rates in twice-open females were not associated directly with the lower systemic progesterone concentrations.

INTRODUCTION

A comparison of plasma LH and progesterone concentrations between cows with normal versus abnormal or no embryonic development indicated cows with normal embryonic development had 1) a shorter interval from onset of estrus to preovulatory LH surge, 2) higher preovulatory LH surge concentrations, and 3) higher plasma progesterone concentrations on Days 3 and 6 after mating (1). Other studies have failed to provide evidence for an association between plasma progesterone concentration and embryonic development before Day 10 after insemination but indicated

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higher progesterone concentrations between Days 10 and 18 in pregnant versus nonpregnant bovine females (2,3,4,5,6). Administration of exogenous gonadotropins at the time of insemination has resulted in larger corpora lutea and inconsistent increases in pregnancy rates in both synchronized and cyclic beef and dairy cows and heifers (7,8,9,10,11, 12). Higher pregnancy rates were attributed to ovulatory and luteinizing functions of the gonadotropins. Thus, observed hormonal asynchronies and/or inadequacies (1,3) may contribute to recovery of a higher percentage of unfertilized oocytes and degenerate embryos, or no recovery of either, from low-fertility beef and dairy females (4,13,14).

The objectives of the present study were 1) to determine the feasibility of increasing pregnancy rates in low-fertility cyclic beef cows and heifers (cows and heifers that were not pregnant after two consecutive breeding seasons) through administration of HCG or GnRH at the time of natural mating, and 2) to evaluate the relationship between systemic progesterone concentrations and embryo survival to Day 30 after mating in high- and low-fertility beef cattle.

MATERIALS AND METHODS

Thirty-eight parous control and 61 twice-open (34 parous cows and 27 nonparous heifers) cyclic beef females were allotted randomly to three pens. Age of control and twice-open cows ranged from four to nine years; twice-open heifers were 30 to 34 months of age. Twice-open females were cyclic but found nonpregnant by palpation after consecutive fall and spring 60-day breeding seasons. Two fertile bulls were placed into each pen of females for 90 minutes twice daily (a.m. and p.m.) and cattle were observed for estrous and mating behavior. Within 30 minutes after natural mating by two bulls, females were assigned randomly by age and fertility to one of three treatment groups and administered intramuscularly either 1) saline (untreated controls), 2) 1000 IU HCG (Sigma, St. Louis, MO), or 3) 100 g GnRH (cystorelin, CEVA Laboratories, Inc., Overland Park, KS). Females remained with fertile bulls for three days after treatment. Jugular vein blood samples were collected by venipuncture at 3, 6, 9, 12 and 18 days after mating for progesterone analysis by radioimmunoassay (15). Blood was collected into heparinized syringes, refrigerated and centrifuged; plasma was stored at -10C until assayed for progesterone. All females were slaughtered 31 to 37 days after mating. Reproductive tracts were collected for gross morphological examination and determination of pregnancy and embryonic development. Corpora lutea were excised from the ovaries and weighed.

Because the contribution of fertilization failure and embryonic mortality to conception losses was reported to differ between parous and nonparous females (16), twice-open cows and heifers were considered separate populations. Effects of treatment and type of female on pregnancy rate were determined by Chi-square analysis. The progesterone data were analyzed by split-plot analysis of variance using least-squares procedures (17). Pregnancy status, type of female, hormone treatment and the two- and three-way interactions were analyzed in the main plot with animals as the experimental unit. The subplot was day plus all of the interactions of day with the effects of the main plot. Because of the large day effect, within day comparisons of treatment, type of female, pregnancy status and their interactions were made by a three-way analysis of variance for unequal subgroups. Means were compared by a least significant difference test. Effects of hormone treatment and type of female on corpus luteum weight and fetal measurement were analyzed in a two-way analysis of variance.

RESULTS AND DISCUSSION

Pregnancy rate. Pregnancy rate at about 33 days after mating was significantly lower in twice-open cows ($P<0.05$) and heifers ($P<0.01$) than in control cows (Table I). These results are consistent with previous reports of lower fertility due to a higher incidence of fertilization failure and embryonic mortality in cows with several previous infertile services (repeat-breeder cows) than in normal cows (4,6,13,14, 18). Data for one control cow, three twice-open cows and one twice-open heifer were excluded from the analyses because of anatomical and pathological abnormalities. Pregnancy rate was lower in twice-open heifers than in twice-open cows; this may be due to the reported lower fertilization rate in nonparous females (16).

TABLE I. PREGNANCY RATES FOR UNTREATED, HCG- AND GnRH-TREATED CONTROL AND TWICE-OPEN FEMALES

Type of female	Treatment	N	Percentage pregnant
Control cows	Untreated	13	76.9
	HCG	12	66.7
	GnRH	12	75.0
	Mean		73.0[a]
Twice-open cows	Untreated	10	60.0
	HCG	11	54.5
	GnRH	10	30.0
	Mean		48.4[b]
Twice-open heifers	Untreated	9	33.3
	HCG	9	33.3
	GnRH	8	37.5
	Mean		34.6[c]

[ab] $P<0.05$
[ac] $P<0.01$

Administration of luteotropic substances at estrus to improve conception rates has provided inconsistent results. In the present study, pregnancy rates were not affected ($P>0.1$) by administration of 1000 IU of HCG or 100 μg of GnRH at estrus or by an interaction of treatment with type of female (Table I). Failure of the HCG treatment to improve pregnancy rate is consistent with results of a larger field trial in which administration of 1500 IU of HCG 12 hr after onset of estrus (time of artificial insemination) had no effect on calving rate in beef cows but tended to lower conception rate in dairy cows (11). Treatment of repeat-breeder dairy cows with HCG at estrus lowered conception rate (10), whereas other studies have indicated that 1500 IU of HCG at the time of artificial insemination improved pregnancy rates 9 to 25% in synchronized and cyclic beef heifers (8,9) and 750 IU of HCG at estrus increased pregnancy rate 15% in cyclic dairy cows (12). The lower rate of recovery of oocytes or embryos from twice-open bovine females may be caused by follicular luteinization rather than follicular rupture and oocyte release. Thus, the luteinizing function of HCG may enhance follicular luteinization rather than correct the lower fertility.

A single injection of 100 µg of GnRH will mediate an LH surge similar to, but shorter than, the preovulatory LH surge in cyclic cows (15,19). The lack of an improvement in pregnancy rate for GnRH-treated twice-open females (Table I) could indicate that delayed LH release, found previously in infertile cows (1,20), was not the predominant factor contributing to lower fertility. Also, monitoring of estrus at 12-hr intervals may have been too infrequent and the endogenous preovulatory LH release had already occurred when GnRH was administered. Alternatively, because of hormonal asynchrony and inadequacies in repeat-breeder cows (14), the GnRH-mediated LH release may have induced follicular luteinization in delayed ovulators, as occurs in therapeutic treatment of ovarian cysts (21), rather than follicular rupture and oocyte release.

Fetal weight and crown-rump length (Table II) were not different (P>0.1) between control and twice-open females or among untreated, HCG- and GnRH-treated females. Therefore, it was concluded that fetal growth was not different among populations and hormone treatment groups.

TABLE II. LEAST-SQUARE MEANS (±SEM) FOR FETAL LENGTH AND WEIGHT AND CORPUS LUTEUM WEIGHT FOR PREGNANT CONTROL AND TWICE-OPEN FEMALES TREATED WITH HCG OR GnRH AT ONSET OF ESTRUS[a]

Parameter	Treatment	n	Type of bovine female: Control	n	Twice-open[b]
Crown rump length (mm)	Untreated	10	14.2 ± 0.9	8	15.0 ± 1.0
	HCG	8	15.3 ± 1.0	9	13.5 ± 1.0
	GnRH	9	13.1 ± 1.0	4	13.5 ± 1.4
Fetal weight (g)	Untreated	10	0.36± 0.16	8	0.37± 0.16
	HCG	8	0.79± 0.16	9	0.33± 0.15
	GnRH	9	0.39± 0.15	4	0.53± 0.20
Corpus luteum weight (g)	Untreated	10	5.65± 0.27	8	5.52± 0.31
	HCG	8	4.94± 0.31	9	5.82± 0.29
	GnRH	9	5.28± 0.29	4	5.60± 0.39

[a] Fetuses and corpora lutea were collected between 31 and 37 days of gestation.
[b] Data were pooled for twice-open cows and heifers.

TABLE III. LUTEAL PHASE PLASMA PROGESTERONE CONCENTRATIONS IN UNTREATED, HCG- AND GnRH-TREATED CONTROL AND TWICE-OPEN FEMALES[a]

Days after mating	Treatment	Control cows				Twice-open cows				Twice-open heifers			
		Pregnant		Nonpregnant		Pregnant		Nonpregnant		Pregnant		Nonpregnant	
		n	Progesterone	n	Progesterone	n	Progesterone	n	Progesterone	n	Progesterone	n	Progesterone
			(ng/ml)		(ng/ml)		(ng/ml)		(ng/ml)		(ng/ml)		(ng/ml)
Day 3	Untreated	11	0.5±0.1	2	0.6±0.3	6	0.3±0.1	4	0.2±0.2	3	0.2±0.2	6	0.3±0.1
	HCG	8	0.7±0.1	4	0.3±0.2	6	0.3±0.1	5	0.4±0.2	3	0.3±0.2	6	0.5±0.1
	GnRH	10	0.4±0.1	2	0.1±0.3	4	0.1±0.2	6	0.3±0.1	3	0.2±0.2	5	0.2±0.2
Day 6	Untreated	11	2.1±0.3	2	2.3±0.6	6	1.4±0.4	4	1.4±0.4	3	1.6±0.5	6	1.3±0.4
	HCG	8	1.7±0.3	4	1.6±0.4	6	1.4±0.4	5	1.3±0.4	3	2.3±0.5	6	2.6±0.4
	GnRH	10	1.5±0.3	2	3.2±0.6	4	1.1±0.4	6	1.1±0.4	3	1.2±0.5	5	1.1±0.4
Day 9	Untreated	11	3.6±0.4	2	3.9±0.9	6	2.2±0.5	4	2.6±0.6	3	2.9±0.7	6	2.3±0.5
	HCG	8	2.8±0.4	4	3.0±0.6	6	3.1±0.5	5	2.0±0.6	3	3.6±0.7	6	4.2±0.5
	GnRH	10	2.5±0.4	2	5.7±0.9	4	2.4±0.6	6	2.3±0.5	3	2.0±0.7	5	2.6±0.6
Day 12	Untreated	11	4.6±0.6	2	3.3±1.4	6	3.0±0.8	4	3.8±1.0	3	2.7±1.1	6	2.6±0.8
	HCG	8	3.4±0.6	4	5.5±1.0	6	4.1±0.8	5	3.4±0.9	3	3.7±1.1	6	5.2±0.8
	GnRH	10	3.2±0.6	2	5.5±1.4	4	2.9±1.0	6	3.2±0.8	3	3.3±1.1	5	2.9±0.9
Day 18	Untreated	11	5.3±0.7	2	3.6±1.5	6	3.6±0.9[b]	4	0.8±1.1[c]	3	4.5±1.6[b]	6	0.7±0.9[c]
	HCG	8	3.5±0.8	4	4.4±1.1	6	3.8±0.9[b]	5	1.2±1.0[c]	3	4.8±1.6	6	4.1±0.9
	GnRH	10	4.0±0.7	2	7.4±1.5	4	2.8±1.1[b]	6	1.9±0.9[c]	3	1.8±1.6[b]	5	0.3±1.0[c]

[a] Mean ± SEM
[bc] Means differ between pregnant and nonpregnant females at $P<0.05$.

Systemic progesterone profile. Mean plasma progesterone concentrations for pregnant and nonpregnant females in the three hormone treatment groups and populations are presented in Table III. Overall plasma progesterone concentrations (Table IV) were significantly lower ($P<0.01$) in twice-open than in control females. However, the treatment x day interaction indicated that populations were not different ($P>0.1$) on Day 12 after mating.

TABLE IV. LEAST-SQUARE MEANS (±SEM) FOR OVERALL PLASMA PROGESTERONE CONCENTRATION IN UNTREATED, HCG- AND GnRH-TREATED CONTROL AND TWICE-OPEN FEMALES

	Treatment					
	Untreated		HCG		GnRH	
Type of female	n	Progesterone	n	Progesterone	n	Progesterone
		(ng/ml)		(ng/ml)		(ng/ml)
Control cows	13	3.0±0.6[a]	12	2.7±0.4	12	3.4±0.4[a]
Twice-open cows	10	1.9±0.4[b]	11	2.1±0.4[c]	10	1.8±0.4[b]
Twice-open heifers	9	1.9±0.5[b]	9	3.2±0.4[d]	8	1.3±0.5[b]

[ab] Means differ among populations at $P<0.01$.
[cd] Means differ among populations at $P<0.05$.
[bd] Means differ among treatments at $P<0.01$.

Although systemic progesterone concentrations were lower in twice-open than in control females, it is doubtful that the reduced fertility resulted from insufficient progesterone since elevated ($P<0.05$) progesterone concentrations in HCG-treated twice-open heifers did not improve fertility. Likewise, treatment of twice-open cows similar to those in the present study with 100 mg of progesterone on Day 2, 3, 4, 6, and 9 of gestation failed to improve fertility (Maurer, unpublished). More critical evaluations of the relationship between plasma progesterone concentration and embryonic development in repeat-breeder bovine females during the first 8 days after insemination have failed to show a difference in progesterone between females with normal and abnormal embryos (4,6). However, the latter studies (4,6) did not include bovine females with unfertilized ovum or no recovery of embryonic tissue, as did the present study. These females may have lower progesterone concentrations than females with abnormal embryos. Investigators who found higher systemic progesterone concentrations in pregnant cows than in nonpregnant cows between Days 9 and 18 after mating attributed the higher progesterone concentrations to the production of an embryonic luteotropic stimulus rather than to lower progesterone concentrations causing embryonic mortality (2,5). The poor success in improving pregnancy rate in medium- and low-fertility bovine females by administration of exogenous progesterone or luteotropic substances during early pregnancy (9,10,22) supports this interpretation. Likewise, progesterone or progesterone + estrone therapy that maintained pregnancy in 73% of the ovariectomized first-service dairy cows maintained pregnancy in only 18% of the ovariectomized repeat-breeder cows (23). Lower plasma progesterone concentrations in twice-open females on Day 3, 6 and 9, but not 12 (Table III), may indicate a delay of the preovulatory LH release and subsequent ovulation and corpus luteum function as reported previously (1,20). Corpus luteum weight (Table II) at slaughter did not differ ($P>0.1$) among populations.

Plasma progesterone concentrations were not different (P>0.1) between pregnant and nonpregnant females (2.35 vs 2.37 ng/ml) from Days 3 through 12 after mating; thus, data for pregnant and nonpregnant females were combined in Figure 1. Also, progesterone did not differ (P>0.1) between nonpregnant and pregnant control cows on Day 18. Our results agree with those of investigators who found no difference in progesterone concentration in blood (4,24,25) or milk (25,26) between pregnant and nonpregnant cows but are contrary to the results of investigators who found higher systemic progesterone concentrations in pregnant cows between Days 9 and 18 of gestation (2,3,5). Discrepancies among experiments as to whether systemic progesterone concentrations differ between pregnant and nonpregnant cows may depend upon the cause of conception loss (fertilization failure versus embryonic mortality) or when embryonic mortality occurred (26,27). Progesterone was reported (27) to be lower on Day 14 after insemination in cows returning to estrus by Day 19 than in pregnant cows but was not different between pregnant cows and cows returning to estrus after Day 19. Also, the functional lifespan of the bovine corpus luteum was extended by the presence of an embryo in the uterus or infusion of a homogenate of 17- and 18-day-old embryos into the uterus between Days 15 and 18 after mating (28). In the present study, a degenerate embryo or necrotic embryonic tissue was found in 19% of the females classified as nonpregnant at slaughter. Thus, the lifespan of the corpus luteum may have been prolonged in these females, which resulted in higher progesterone concentrations for the nonpregnant groups during the evaluation period.

In contrast to control cows, Day 18 plasma progesterone concentrations were lower (P<0.05) in nonpregnant than in pregnant twice-open females except in HCG-treated heifers (Table III). Lower progesterone concentrations on Day 18 in nonpregnant twice-open females than in nonpregnant control females may suggest premature regression of the corpus luteum in twice-open females due to insufficient embryonic and/or pituitary luteotropic stimulation or decreased capacity of the luteal tissue to respond. Again, cause and time of conception loss may influence the progesterone concentrations of nonpregnant bovine females during the first 18 days after insemination, especially when pregnancy rate is not determined until after 30 days of gestation.

Treatment of twice-open heifers with 1000 IU of HCG at estrus increased (P<0.05) plasma progesterone concentrations (Table IV) during the subsequent luteal phase and may have extended the estrous cycle (Table III). However, the HCG treatment had no effect on corpus luteum function in control and twice-open cows (Table IV). A previous study in which 1500 IU of HCG at estrus increased corpus luteum size and function was also conducted in cyclic heifers (7); thus, the luteotropic response to HCG may be affected by parity. Treatment of cows with 100 μg of GnRH at estrus did not affect (P>0.1) systemic progesterone concentrations in either twice-open or control females (Table IV). Corpus luteum weight was not different among the three hormone treatment groups: untreated, 5.59 ±0.20 g; HCG, 5.40 ±0.21 g and GnRH, 5.40 ±0.23 g. Again, the lack of either an HCG or a GnRH treatment effect on systemic progesterone concentration, corpus luteum weight and fertility may indicate that the hormones were 1) administered at an inappropriate time, 2) ineffective in hastening the preovulatory LH surge, ovulation and corpus luteum formation, 3) the cause of

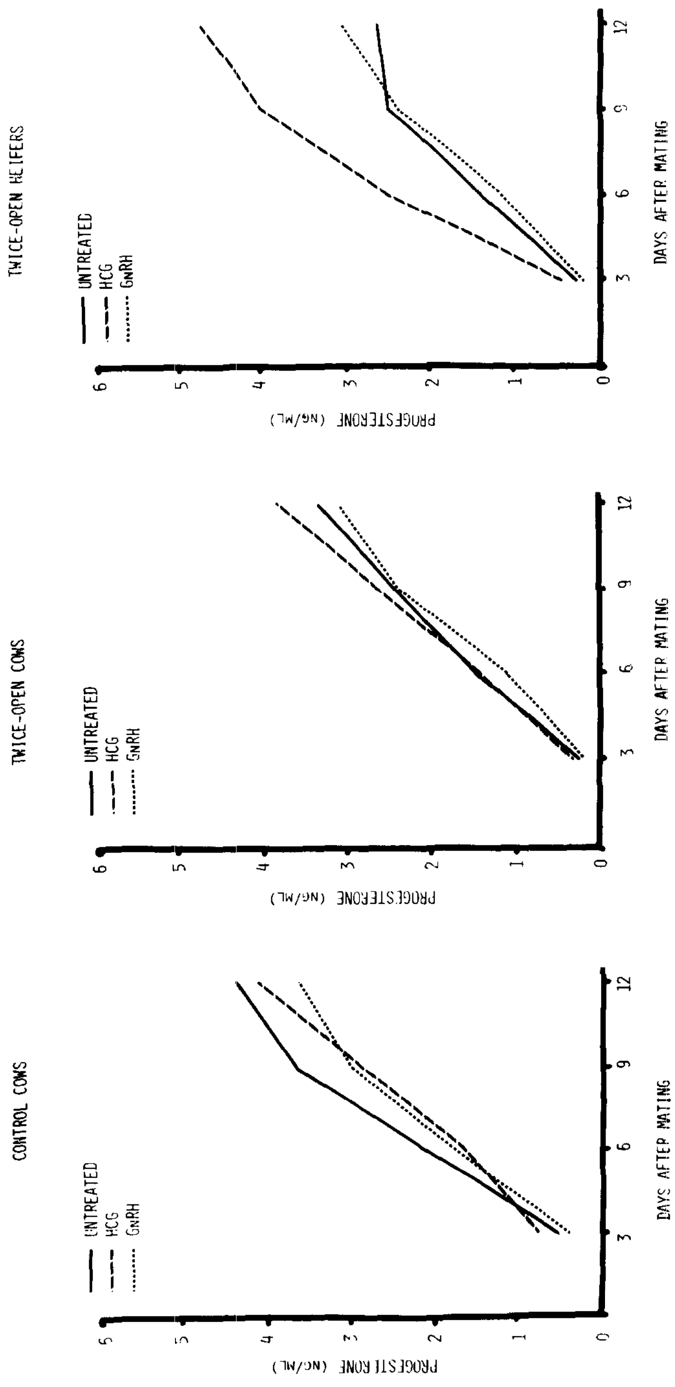

Figure 1. Plasma progesterone profiles for Days3 through 12 after mating in untreated, HCG- and GnRH-treated control cows and twice-open cows and heifers. Data were pooled for pregnant and nonpregnant females.

follicular luteinization rather than follicular rupture and oocyte release, or 4) a combination of these factors. Because of the high incidence of no recovery of embryo or oocyte in twice-open bovine females, the contribution of abnormal luteinization of the ovarian follicle and release of the oocyte from the follicle needs to be further investigated.

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